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An evolutionary perspective on the resistance of *Daphnia* to the epizoic rotifer *Brachionus rubens*

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SUMMARY

1. In considering the evolutionary dynamics of host–parasite interactions, it is usually assumed that host populations are able to evolve rapidly under selection from various parasitic pressures. This has been shown for endoparasites, but for ectoparasites and/or epibionts, in general, less strong effects are expected on their hosts.

2. We show that the exposure of *Daphnia magna* clones (of a different origin) to lake sediment from the Danish Lake Ring, containing dormant eggs of the epizoic rotifer *Brachionus rubens*, results in infections that are associated with increased mortality in *Daphnia*.

3. Using a resurrection ecology approach, we reconstructed evolutionary change in the Lake Ring *D. magna* population in response to *B. rubens* infections. We observed interclonal variation in resistance to *B. rubens* in this *Daphnia* population, as well as changes over time in resistance to rotifers.

4. Both sediment exposure and time period from which Lake Ring clones were isolated affected important life-history parameters, suggesting that evolution (upon exposure to *Brachionus*) not only affected resistance to *Brachionus*, but also other traits in Lake Ring *Daphnia*.

5. Additionally, there were complex effects of a joint exposure of *D. magna* to rotifers and the indirect effect of fish predation: exposure to fish kairomones resulted in an increased allocation of energy to early reproduction and *Daphnia* performance. Further, fish kairomones apparently increased *B. rubens* infectivity.

Keywords: *Daphnia magna*, epizootic rotifer, micro-evolution, resurrection ecology

Introduction

Host populations are under selection from parasite infections, and parasites are constantly evolving with respect to their capacity to overcome host defences and to invade host individuals. This can lead to adaptive genetic changes both in the host and in its (endo)-parasites (Woolhouse *et al.*, 2002; Ebert, 2008; Penczykowski, Forde & Duffy, 2011). Such dynamics of adaptive processes have been well documented in *Daphnia* for endoparasites (Decaestecker *et al.*, 2007; Wolinska *et al.*, 2011). By definition, endoparasites develop internally in the host and, in general, sequester resources from it, inducing strong negative effects on host fecundity and survival.

Epibionts live on the surface of the host and are usually considered harmless or even beneficial. In general, they are expected to have a smaller impact on the host population than endoparasites (Decaestecker *et al.*, 2005; Ebert, 2005), but negative effects for epibionts have been reported (Green, 1974; Willey, Cantrell & Threlkeld, 1990; Chiavelli, Mills & Threlkeld, 1993). Rotifers live in close association with other organisms (May, 1989). They may compete strongly with the host for food (Matveeva, 1989). Furthermore, because rotifers are attached to the carapace of their host, they can reduce zooplankton

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buoyancy and host mobility, making it more difficult for the host to obtain food, thus constraining survival, growth and fecundity (Matveeva, 1989; Iyer & Rao, 1993). Jaramillo-Londono & Pinto-Coelho (2010) reported that intrusion of individuals of the rotifer *Hexartha intermedia* into the filtering chamber of the cladoceran *Bosmina longirostris* revealed signs of a new ecological relationship that could be seen as a fusion of parasitism with interference competition. Here, we investigated whether there is a potential for evolutionary responses by *Daphnia* as a result of the selective pressure of epizoic rotifers.

Brachionus rubens is the most widespread and common rotifer that lives facultatively on cladocerans. It can have a planktonic stage but is commonly epizoic and attached to the carapax of large-bodied *Daphnia* via a secretion released by the pedal glands (Green, 1974). Through a behavioural adaptation for rapid attachment (Iyer & Rao, 1993), *B. rubens* minimises the effects of mechanical interference, which occurs when rotifers are swept into the *Daphnia*'s branchial chamber and are ingested, or more commonly, damaged when rejected (Dieguez & Gilbert, 2011). This enables the rotifers to cover the entire surface of the *Daphnia. Brachionus* infections clearly can be harmful to the *Daphnia* host. If the large number of *Brachionus* individuals form a 'sleeve' around the host, it can lead to complete immobility and mortality in *Daphnia* juveniles (Matveeva, 1989). *Brachionus* infections reduce the swimming capacity and feeding rate of *Daphnia* (Iyer & Rao, 1993), possibly resulting in stress and trade-offs in energy allocation in the *Daphnia* and causing negative effects on *Daphnia* survival, growth and fecundity.

To trace evolutionary changes in the epizoic–host interaction, we used a 'resurrection ecology' approach (Hairston *et al*., 1999; Cousyn *et al*., 2001; Jeppesen *et al*., 2001; Kerfoot & Weider, 2004). In freshwater systems, the long viability of dormant stages of zooplankton, and the fact that they are deposited in stratified, dateable lake sediments, allows comparison of traits from hatched individuals derived from different time periods (Decaestecker *et al*., 2004; Jansen *et al*., 2010; Frisch *et al*., 2014). It is possible to confront hatched *Daphnia* clones with infective stages and thus to reconstruct evolutionary changes in adaptation between antagonists over time (Decaestecker *et al*., 2007). In our study, we used this approach to reconstruct changes in *Daphnia* resistance to infection with the epizoic rotifer *Brachionus rubens* in the natural *D. magna* population of Lake Ring, Denmark.

We hypothesised that the *Daphnia*–epizoic interactions are embedded in a broader set of interactions within the community and focussed on fish predation. Predation is important in the ecology and evolution of zooplankton, and causes both direct and indirect effects. Predator induced changes in behaviour or energy allocation can cause strong effects with respect to micro-evolutionary interactions of prey populations (Cousyn *et al*., 2001; Decaestecker, De Meester & Ebert, 2002; Preisser, Bolnick & Benard, 2005; Beckerman, Wieski & Baird, 2007). Here, we addressed only indirect effects, partly due to logistical constraints but also because experimental exposure of *D. magna* to fish kairomones has shown there is microevolution of antipredator traits in response to fish predation in Lake Ring (Michels *et al*., 2007). These effects may have indirectly affected *Brachionus* infectivity through changes in *Daphnia* life history.

We performed experiments to reconstruct the dynamics of abundance of the epizoic rotifer *B. rubens* in the *D. magna* population of Lake Ring. We also investigated whether responses to *Brachionus* have been indirectly modified by fish presence. Our particular aims were to test

1. for the potential of *D. magna* to evolve resistance to *B. rubens.*
2. whether changes in *D. magna* over time were associated with changes in the density of *B. rubens.*
3. whether the response of the resident *Daphnia* population to the rotifer has been affected by the presence of predator cues (fish kairomones).
4. if the costs of adaptation (reflected in fecundity parameters) to rotifer infection and the presence of fish kairomones have changed over time.

Methods

Study site and Daphnia clones

Lake Ring is a shallow lake (average depth 2.9 m; 22.5 ha) located in central Jutland (Denmark, 55°57′51.83″N, 9°35′46.87″E). The lake became strongly eutrophic at the beginning of the 20th century, due to sewage inputs. In an effort to restore the ecosystem, the sewage inflow was stopped in 1970. This first led to a very gradual recovery, followed by a substantial increase in water clarity from the mid-1980s onwards (for more details and a description of Lake Ring, see Michels *et al*., 2007). Three successive periods in the history of Lake Ring can be discerned: clear water from 1908 to 1937 and from 1986 to 2000 (henceforth referred to as the pre-eutrophication and the clear water periods) with a turbid period (1960–1980), characterised by high fish predation, between the two. The lake has long received untreated sewage from the City Brædstrup

(*c.* 3.500 inhabitants) and, between 1937 and 1960, the lake gradually became more eutrophic. Periodic fish kills, typical for lakes receiving high organic matter loading, is probably the reason for the gradual increase in the abundance of *D. magna*. The paleolimnological data, however, also indicated an overall increase in fish predation pressure, suggesting that periods with low fish predation (after fish kills), with dominance of *D. magna* and relatively clear water, alternated with periods dominated by smaller cladocerans and with turbid water. This is often the case in other hypertrophic lakes with high loading by untreated sewage water (Jeppesen *et al.*, 1998). We identified the study periods both (i) to be sure that we had clearly defined periods in which we knew that the conditions were distinct and (ii) from which we could hatch sufficient clones, which is why we allowed a longer intervening period (1937–60) for which there were less historical data.

Three sediment cores (length: 30 cm, diameter 15 cm) were taken from Lake Ring and sectioned horizontally into 0.5-cm slices, packed in light-tight containers and stored at 4 °C until further processing. *Daphnia magna* clones were hatched from the cores by filtering the sediment through a 250-μm mesh and exposing it to hatching stimuli (20 ± 1 °C, fresh medium, 16L:8D photoperiod). Six, 11 and nine clones were successfully hatched from the pre-eutrophication, the turbid and the clear water periods, respectively (Michels *et al.*, 2007), and were kept as clonal lineages in the laboratory for several generations before the experiment.

Experimental design

We conducted two successive experiments under standardised laboratory conditions (20 ± 1 °C, medium refreshed daily, 16L:8D photoperiod).

In Experiment 1, we exposed naïve Belgian *Daphnia* clones to Lake Ring sediments from three different depths in the core, corresponding to the three distinct time periods. This allowed us to measure the impact of *B. rubens* infection on *Daphnia* and to reconstruct the abundance of *B. rubens* in Lake Ring for the different time periods, under the assumption that the number of dormant stages reflects the density of rotifer populations at the time (Verschuren & Marnell, 1997; Jeppesen *et al.*, 2001; Decaestecker *et al.*, 2004) and that viability of the dormant stages does not decline over time (as in Decaestecker *et al.*, 2004).

In Experiment 2, we tested whether there were differences in resistance to *B. rubens* among the *Daphnia* clones derived from the different time periods in Lake Ring. In addition, we tested whether rotifer resistance to infection was influenced by the indirect response to predation risk. Therefore, *D. magna* clones derived from different time periods (hence different fish predation regimes) were exposed to Lake Ring sediment in the presence and absence of fish kairomones.

In Experiment 1, we used four random *Daphnia* clones, isolated from sediments of a natural pond [Oud Heverlee, Belgium, 50°50′22.16″N, 4°39′18.16″E; clones B1, B4, B6 en B8 in Cousyn *et al.* (2001)] with a low concentration of dormant *Brachionus* eggs (Pauwels *et al.*, 2010). We used four clones to counter any clone-specific responses and used clones that did not originate from Lake Ring to avoid any interference caused by adaptation of the local clones to possible (ecto-)parasites. We assumed that more than one endo- or ectoparasitic species could be present in the Lake Ring sediment, but detected mainly infections with the epizoic rotifer *B. rubens*. At the start of the experiment, a cohort of seven newborns (younger than 24 h) of each *Daphnia* clone were jointly exposed to Lake Ring sediment from each of the three different time periods (exposure in 50-mL jars, containing about 5 mL of mixed sediment from a given time period and 45-mL dechlorinated tap water) in nine replicates (in total: four clones x three time periods x nine replicates: 108 experimental jars). During the first 3 days, the experimental *Daphnia* were fed daily a relatively small amount of food (1×10^5 algal *Scenedesmus obliquus* cells mL^{-1}, corresponding to 0.75 mg C L^{-1}) to stimulate benthic grazing behaviour to increase contact with the sediment and thus the likelihood of infection with the parasites and (hatching) epibionts present (Decaestecker *et al.*, 2002). Subsequently, the food was doubled to increase parasite growth (Pulkkinen & Ebert, 2004). At normal temperatures, *Brachionus* resting eggs typically hatch within a few days, juveniles mature 2 days later and, in optimal conditions, can lay eggs up to three times a day, which can lead rapidly to high densities (Gilbert, 2004). We scored *Daphnia* for infection with adult *B. rubens* after 7 days of exposure to lake sediment. We measured prevalence (proportion of infected individuals), infection intensity (mean number of *B. rubens* per *Daphnia* individual, corrected for dry mass of the sediment) and *Daphnia* survival. The relative short exposure time and the fact *B. rubens* attaches preferentially to living hosts (Iyer & Rao, 1993) ensures that *Brachionus* infection intensity can be used as a good proxy for the relative initial density of resting eggs in the sediment. We note that we might have overestimated actual prevalence, compared with the field situation, given that our experiment involved

an artificially high exposure to the sediment in a small volume of water. However, our experimental exposure was performed as an assay to quantify changes in rotifer abundance over time as well as the intrinsic capacity of *Daphnia* clones to deal with *Brachionus*, rather than as a mimic of natural conditions.

In Experiment 2, we studied the resistance to *B. rubens* of the *Daphnia* clones derived from the different time periods. We reared all clones for several generations in standardised conditions prior to the experiments. At the start of the experiment, we exposed the *Daphnia* clones from the different sediment depths to a sediment suspension consisting of a mixture of 60 core slices covering the three different time periods or to a control treatment (without sediment). In total, there were 312 experimental jars (two sediment treatments × two fish treatments × 26 clones in three replicates). We added 400 mL of standardised sediment suspension to the experimental jars in the sediment suspension treatment. When settled, these contained 28 cm^3 of sediment. The control treatment consisted of 400 mL dechlorinated tap water. We allowed the sediment to settle for 1 day before inoculating the *Daphnia*. Subsequently, in both sediment and control jars, the medium was diluted by adding 100 mL fish medium or dechlorinated tap water, depending on the treatment (presence or absence of fish kairomones). Fish medium was prepared as described in Decaestecker *et al.* (2002): dechlorinated tap water was conditioned by the presence of three *Leuciscus idus* (*c.* 6 cm) in a 20 L aquarium for 24 h, and filtered through a 0.45-μm mesh. Fish were fed outside the aquarium to avoid interference from alarm substances from their prey. The final concentration of fish kairomones in the experimental jars was equivalent to three fish per 100 L. At the start of the experiment, each jar was inoculated with five newborns (younger than 24 h) of a single *Daphnia* clone. Every day, 20% of the medium was refreshed with either fish-conditioned or aged tap water, and all juveniles were removed.

On day 22, all individuals were checked visually for *B. rubens* presence. In addition, two of the five animals per experimental jar were dissected and thoroughly examined for the presence of other parasites using phase-contrast microscopy at 400× magnification. This provided data on parasite infection intensity for both *B. rubens* and other parasites. In addition to mortality, we also quantified life-history parameters on all animals in the experiment. We measured age at release of first brood and fecundity (total number of offspring produced during the course of the experiment: 22 days corrected for surviving *Daphnia*). As a measure of performance, we calculated the intrinsic population growth rate 'r' (based on the number of offspring and survival during the experiment, following the Euler equation; see Stearns, 1992).

Data analysis

In Experiment 1, we investigated parasite density over time in Lake Ring by exposing naïve *Daphnia* clones to different lake sediment depths and investigating parasite infection intensity. As variances were not homogeneous during the different time periods, we fitted the data into a generalised least square (GLS) model to analyse the time period effect of the Lake Ring sediment as well as the clonal variation on *B. rubens* infection intensity and *Daphnia* survival. To investigate whether *B. rubens* infection intensity was associated with fitness effects in the *Daphnia* host, we performed a Spearman Rank Correlation test between *B. rubens* infection intensity and *Daphnia* survival, using *Daphnia* clonal means.

In Experiment 2, we analysed resistance to *B. rubens* with respect to infection intensity, mortality and impact on fecundity and age at first brood in the resident *D. magna* population of Lake Ring. This was investigated in the absence and presence of fish kairomones. *Brachiouns rubens* infection intensity was analysed using a general linear model (ANOVA) testing for the effect of clone (random factor nested in time period), time period, fish kairomones and their interaction effects. Fecundity parameters were similarly analysed using a general linear model (ANOVA), with clone (random factor nested in time period), time period, fish kairomones and the presence or absence of infectious sediment as factors. The generalised least square (GLS) models were fitted by REML, R package, version 2.15 (R Foundation for Statistical Computing, Vienna, Austria), whereas all other statistical analyses were performed using STATISTICA 9.1 (StatSoft, Tulsa, OK, U.S.A.).

Results

In both experiments, exposure of *Daphnia* to sediment from Lake Ring resulted in infections by the epizoic rotifer *B. rubens*. Overall, we observed an overall mean of >60% prevalence for both experiments. Sporadically, an unknown brood parasite infection was also recorded, this being minor (mean over both experiments: 4%) and was therefore excluded from further analyses.

The prevalence of the rotifer infections, as well as the number of rotifers on the naïve Belgian *Daphnia* clones, decreased upon exposure to more recent Lake Ring sediment (Experiment 1; Fig. 1a). This was associated with a

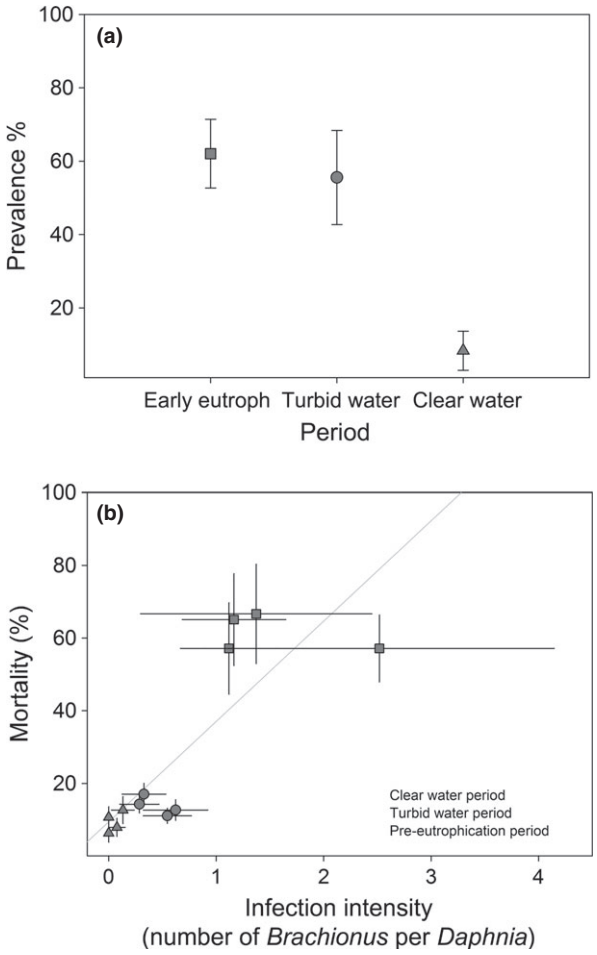

Fig. 1 Results of Experiment 1. (a) Prevalence (proportion of infected cohorts) in four *Daphnia* clones from a Belgian pond (Oud Heverlee) exposed for 7 days to *Brachionus rubens* dormant eggs present in sediment from the three different time periods of Lake Ring. (b) Clonal means of *Daphnia* survival plotted against infection intensity (average number of *Brachionus* on *Daphnia* individuals, corrected for dry mass of the sediment). Means ± SE and the regression line are given; symbols: squares, circles and triangle represent the pre eutrophication (1908–37), turbid water (1960–80) and clear water (1986–2000) periods, respectively.

significant effect of time period on *B. rubens* infection intensity (GLS test, time period effect: $F_{2,85} = 8.08$; $P < 0.001$). *Daphnia* mortality also decreased when *Daphnia* individuals were exposed to sediment from more recent versus older time periods (Fig. 1b, GLS, time period effect: $F_{2,96} = 61.4$; $P < 0.001$). No clonal variation in either prevalence or survival in the Belgian clones was observed (GLS, clone effect: $F_{3,85} = 0.85$; P = ns; $F_{3,96} = 0.23$; P = ns). The Belgian clones also did not respond differently upon exposure to *Brachionus* containing mud from different time periods in Lake Ring (GLS, clone × time period interaction: $F_{6,85} = 0.39$; P = ns; $F_{6,96} = 0.26$; P = ns). Across clones, there was a strong negative correlation between *Daphnia* survival and *B. rubens* infection intensity (Spearman Rank on averages for all clone and time period combinations; $n = 12$; $r = -0.85$; $P = 0.0005$, Fig. 1b), suggesting that increasing numbers of *B. rubens* individuals had a cumulative negative effect on *Daphnia* survival.

In Experiment 2, we observed a strong *Daphnia* clone effect on *B. rubens* infection intensity (Table 1), indicating high variation in parasite resistance in the set of Lake Ring *Daphnia* clones studied. Time period had a significant effect on *B. rubens* infection intensity (Fig. 2; Table 1). A Fisher LSD *post hoc* test indicated that *B. rubens* had a significantly lower infection intensity in *Daphnia* clones derived from the recent clear water period than in clones derived from the older turbid period (mean *B. rubens* infection intensity clear water period 14.95 ± 1.81 versus turbid water period 21.31 ± 1.66, $P < 0.005$). There was no significant difference between pre-eutrophication and the clear water period, or between pre-eutrophication and turbid water period. Exposure to fish kairomones tended to increase infection intensity (marginally non-significant by ANOVA, $P = 0.06$: Table 1; Fig. 2).

There was a strong increase in mortality (Table 2; Fig. 3a), a reduction in age of first brood (Table 2; Fig. 3b), and a decrease in total number of offspring (Fig. 3c), in the Lake Ring clones upon exposure to the lake sediment containing *Brachionus*. These different responses in fitness traits equalled each other, such that there was no significant effect of sediment on *Daphnia* performance (Table 2; Fig. 3d). The increase in mortality and the decrease in total number of offspring and performance in the presence of sediment was clone-dependent (see sediment × clone interaction in Table 2).

Further analysis of *Daphnia* life-history traits revealed strong clonal effects (Table 2). Time period had no significant effect on *Daphnia* mortality (Table 2; Fig. 3a), but there was a significant time period effect on the age

Table 1 General linear model testing for the effect of clone (random factor nested in time period), time period and fish kairomones on *Brachionus rubens* infection intensity on *Daphnia magna* clones hatched from sediments from three different time periods in Lake Ring (Experiment 2)

Source of variation	Effect	d.f.	*F*	*P*
Clone (time period)	Random	23, 95	**2.77**	**<0.001**
Time period	Fixed	2, 95	**3.38**	**0.04**
Fish kairomones	Fixed	1, 95	3.50	0.06
Clone (time period) × Fish kairomones	Random	23, 95	0.63	0.96
Fish kairomones × Time period	Fixed	2, 95	0.04	0.89

Significant *P*-values are indicated in bold.

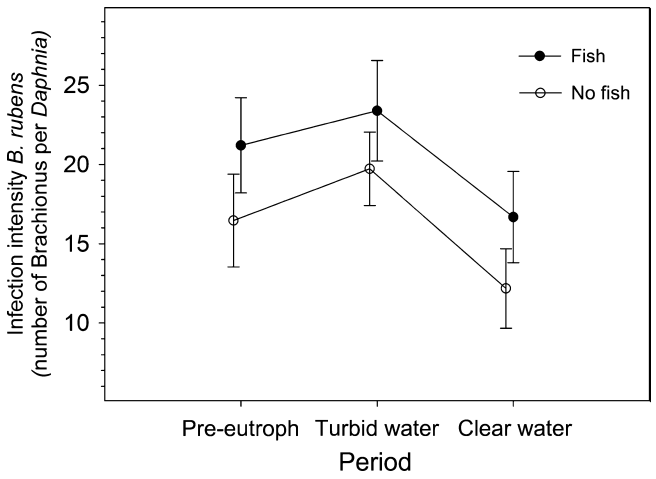

Fig. 2 *Brachionus rubens* infection intensity after 22 days in the presence (solid symbols) and absence (open symbols) of fish kairomones on *Daphnia* clones for the three populations from different time periods in the history of Lake Ring in Experiment 2. Population means ± SE were calculated using clonal means.

at release of first brood and on fecundity (Table 2). *Daphnia* from the recent clear water period released their brood at a later age than did those from the two older Lake Ring time periods (the pre-eutrophication and turbid water periods, Fig. 3b; Fisher LSD *post hoc* test; mean age at release of first brood of clones from the pre-eutrophication period 10.8 ± 0.66 and from the turbid water period 10.8 ± 0.12 versus clones of the clear water period 11.2 ± 0.14, with $P = 0.02$ and 0.05, respectively). *Daphnia* from the recent clear water period also produced less offspring than clones from the older time periods (Fig. 3c: Fisher LSD *post hoc* test; mean fecundity of clones of the pre-eutrophication period 17.7 ± 0.51 juveniles/*Daphnia* and from the turbid water period 16.9 ± 0.42 versus clones of the clear water period 15.8 ± 0.45, with $P < 0.001$ and $P = 0.02$, respectively). There was no significant effect of time period on performance ('little r': Table 2; Fig. 3d).

Exposure to fish kairomones did not affect mortality (Table 2; Fig. 3a), but fish exposure reduced the age at release of first brood (Table 2; Fig. 3b) and increased *Daphnia* fecundity (Table 2; Fig. 3c), which resulted in superior *Daphnia* performance in the presence of fish kairomones (Table 2; Fig 3d).

Discussion

Using a resurrection ecology approach, we analysed changes in the abundance of the epizoic rotifer *B. rubens* and its impact on *D. magna* over time. These changes took place over the course of the last century in a natural lake that has a well-documented history of eutrophication and recovery, and associated changes in fish predation response in *Daphnia* (Lake Ring, Denmark: Berg *et al.*, 1994; Jeppesen *et al.*, 2000; Michels *et al.*, 2007). With respect to changes in *B. rubens* density over time, our observations of a dense dormant egg bank of rotifers in Experiment 1 suggest that *B. rubens* population density was much greater formerly (1908-1937) than more recently. This conclusion relies on the assumption that the number of dormant eggs reflects population density and that the viability of eggs does not decline over time, as discussed in Decaestecker *et al.* (2004). However, note that if dormant eggs were less viable with ageing, our interpretation is conservative, as this would imply that the dormant egg bank in older

Table 2 General linear model testing for the effect of clone (random factor nested in time period), time period, fish kairomones and sediment containing *Brachionus rubens* on mortality, age at first brood, fecundity and performance of *Daphnia magna* hatched from sediments from three different time periods in Lake Ring (Experiment 2)

			Mortality		Age at first brood		Fecundity		Performance	
Source of variation	Effect	d.f.	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Clone (Period) C(P)	Random	23, 207	0.89	0.61	**4.15**	**<0.001**	**4.10**	**<0.001**	**3.29**	**0.03**
Time period (P)	Fixed	2, 207	0.02	0.98	**6.23**	**0.002**	**6.87**	**0.001**	0.80	0.46
Fish kairomones (F)	Fixed	1, 207	0.08	0.78	**5.86**	**0.018**	**19.3**	**<0.001**	**57.5**	**<0.001**
F × C (P)	Random	23, 207	1.24	0.30	1.32	0.16	0.61	0.92	0.47	0.96
F × P	Fixed	2, 207	1.20	0.32	0.60	0.55	0.18	0.84	0.10	0.90
Sediment (S)	Fixed	1, 207	**14.1**	**<0.001**	**10.7**	**0.001**	**108**	**<0.001**	2.37	0.14
S × C(P)	Random	23, 207	**2.57**	**0.01**	0.68	0.87	**1.89**	**0.01**	**2.06**	**0.04**
S × P	Fixed	2, 207	0.59	0.56	0.87	0.42	1.16	0.31	0.30	0.74
Sediment × Fish kairomones	Fixed	1, 207	0.90	0.35	0.00	0.96	0.08	0.78	0.15	0.70
S × F × C(P)	Random	23, 207	0.45	0.99	0.73	0.81	1.03	0.43	0.92	0.57
S × F × P	Fixed	2, 207	1.24	0.31	1.02	0.36	0.21	0.81	0.52	0.60

Significant *P*-values are indicated in bold.

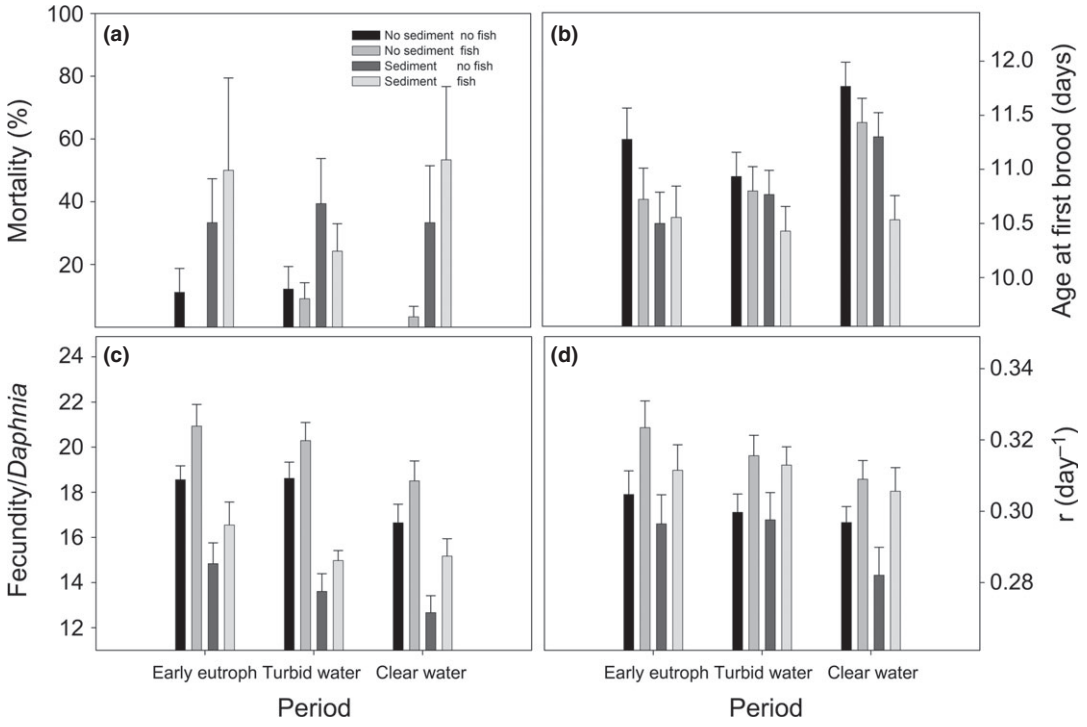

Fig. 3 (a) mortality (% dead at the end of the 22 day experiment, (b) age at first brood, (c) fecundity (total number of offspring/*Daphnia* produced in 22 days), and (d) performance of *Daphnia* clones hatched from sediments representing three different time periods in Lake Ring, in the presence and absence of fish kairomones and sediment containing *Brachionus rubens* in Experiment 2. Means ± SE are given.

sediments is even denser than observed. Another assumption is that infections after 7 days are a good proxy for the density of dormant eggs in the sediment and that there are no differences in the reproductive capacity of rotifers hatched from different time periods. A difference in rotifer reproduction seems rather unlikely, given that we would expect a greater reproduction in the intermediate, eutrophication period than in the oldest, pre-eutrophication period (Jaramillo-Londono & Pinto-Coelho, 2010). Nevertheless, there was no difference in prevalence (estimated population density of rotifers) between the eutrophication and pre-eutrophication periods, and infection intensity was much lower in the former than the latter. Such a difference in rotifer reproduction would again make the observed differences in infection intensity more conservative.

Most studies on the impact of epibionts report relatively small effects on *Daphnia* populations (Decaestecker *et al*., 2005; Ebert, 2005), with the exception of Kankaala & Eloranta (1987) who reported a strong competitive effect of *Vorticella* on *Daphnia longispina*. Nevertheless, we show here that *Brachionus rubens* may exert selection pressure on *Daphnia* in several ways. Exposure of *Daphnia* to the sediment (containing dormant eggs of the epizoic rotifer) resulted in severe mortality (increased by up to >35%) and reduced fecundity. As both antagonists feed on 'edible' algae, the rotifers are likely to compete with *Daphnia* for food (Gilbert, 1988). By attaching to the carapace of its host, *B. rubens* can interfere with the *Daphnia* filter apparatus, reducing its feeding rate. High burdens of rotifers on zooplankton individuals and associated negative effects have been reported in the field and may occur more often than one would expect from the literature, given that detection is difficult since the rotifers often detach very quickly upon sampling. Intrusion of rotifers into the filtering chamber of the host has been described as a mixture of parasitism with interference competition, increasing rotifer fitness, especially in eutrophic conditions (Jaramillo-Londono & Pinto-Coelho, 2010).

We cannot fully exclude the possibility that the negative effects of the rotifers on *Daphnia* were the result of sediment toxicity, but this seems unlikely for the following three reasons. First, if toxic effects occurred, we would have expected these to be strongest in the turbid, intermediate period, when the lake received sewage, whereas the most notable effect of the sediment with rotifers to naïve *Daphnia* was found in the pre-eutrophication period in Experiment 1. Secondly, in the presence of sediment,

Daphnia matured more quickly, an effect that cannot be attributed to sediment toxicity. Thirdly, a cumulative negative effect of *B. rubens* individuals on host mortality was observed, an effect that was reflected in the negative correlation of *B. rubens* infection intensity with *D. magna*.

In agreement with the strong impact of *B. rubens* infection on *Daphnia* fitness, we observed significant variation (presumably genetic) in the resistance to *Brachionus* infection of the *D. magna* population of Lake Ring in Experiment 2. When exposing different *Daphnia* clones hatched from the dormant egg bank of this lake to sediments containing dormant eggs of *B. rubens*, strong differences emerged in infection intensity among clones. This clonal variation in infection intensity reflects evolutionary potential and was observed both in the presence and absence of fish kairomones. Our data revealed a change over time in how the *Daphnia* population coped with *B. rubens* infectivity, as *Daphnia* clones derived from the most recent, clear water period (from 1986 onwards) were less infected with *Brachionus* than the clones derived from the preceding time periods. Our data thus suggest micro-evolution with respect to rotifer resistance over time, which is probably the result of a combination of competitive strength and defence mechanisms (behavioural adaptations which may result in avoiding rotifers, see Gilbert & Hampton, 2001; or increased mechanical interference, where rotifers are damaged or killed as they are swept into the filter chamber and are then further ingested, see Gilbert, 1988). It is possible that the increased resistance may have contributed to the decline in *Brachionus* abundance observed in the most recent period.

The evolved defence was, however, associated with reduced fecundity (delayed reproduction and a lower total number of offspring) in *Daphnia* from the recent clear water period in Lake Ring, which might possibly reflect a cost related to their resistance to *Brachionus* during that time period. However, the release from eutrophication could also have had a strong impact on the difference in fecundity parameters between the time periods. When more resources are available, traits associated with higher growth and reproduction are mostly favoured (Tessier, Leibold & Tsao, 2000), influencing the competitive strength of the *Daphnia*. This could also explain the reduced fecundity in the clear water period, although it contrasts with the high fecundity characteristic of the pre-eutrophication period, which was also nutrient depleted (at least relative to the eutrophication period).

We observed the typical indirect effects of fish predation, when Lake Ring *Daphnia* were exposed to fish kairomones: an increased allocation of energy to early reproduction that positively affected *Daphnia* fecundity and performance, even in the presence of sediment containing *B. rubens*. Although rapid development, associated with a greater number of, and a shorter interval between, moultings might potentially release the *Daphnia* from rotifer infection on their carapaces (Threlkeld, Chiavelli & Willey, 1993), exposure to fish kairomones tended to increase *B. rubens* infection intensity. This increased infection intensity might be the result of behavioural shifts (as detected for this population by Michels *et al*., 2007), causing increased encounter rates with *Brachionus*. Alternatively, it could reflect reduced investment in parasite resistance, as a competing cost with investment in antipredator responses (Yin *et al*., 2011). Further, as rotifers suffer from mechanical interference (Gilbert, 1988), the increase in *Brachionus* infection intensity upon exposure to fish kairomones might be the result of a reduction in filtering rate and energy uptake of *D. magna* induced by fish (Beckerman *et al*., 2007), or another behaviour that might increase the propensity of *Brachionus* to attach. The presence of fish kairomones resulted in a higher performance (a faster population growth) and might indirectly have contributed to the decline in rotifer abundance in the Lake Ring population over time, because of changes in competitive interactions. Note, however, that we did not measure the direct effect of fish predation on *Daphnia* mortality, so we cannot disentangle any effect on mortality of an interaction between fish predation and the number of rotifers on the *Daphnia*.

In conclusion, our results revealed temporal changes in the density of the rotifer *B. rubens* in Lake Ring, with the highest population density in the older sediment layers. *Brachionus rubens* infection intensity was found to be strongly associated with *Daphnia* mortality, indicating that *B. rubens* might act as a strong selection pressure, which differs with what is generally assumed for epizoic rotifers or the ectoparasites of zooplankton in general. In line with *B. rubens* being a selection pressure, we observed variation in resistance against *B. rubens* of the resident *D. magna* population of Lake Ring. This micro-evolutionary potential translated into changes over time, with Lake Ring *Daphnia* from the most recent clear water period showing lower infection intensity compared with those of earlier time periods. Both sediment exposure and age of the sediments from which the clones were isolated affected important life-history parameters, suggesting that evolution has not only affected resistance to *Brachionus*, but also other traits in the Lake Ring population of *D. magna*. All these effects cannot be considered independently of indirect effects of fish kairomones,

given that there was a strong increase in performance, differing among clones, upon exposure to fish kairomones.

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References

Beckerman A.P., Wieski K. & Baird D.J. (2007) Behavioural versus physiological mediation of life history under predation risk. *Oecologia*, **152**, 335–343.

Berg S., Jeppesen E., Søndergaard M. & Mortensen E. (1994) Environmental effects of introducing whitefish, *Coregonus lavaretus* (L), in Lake Ring. *Hydrobiologia*, **275**, 71–79.

Chiavelli D.A., Mills E.L. & Threlkeld S.T. (1993) Host preference, seasonality, and community interactions of zooplankton epibionts. *Limnology and Oceanography*, **38**, 574–583.

Cousyn C., De Meester L., Colbourne J.K., Brendonck L., Verschuren D. & Volckaert F. (2001) Rapid, local adaptation of zooplankton behavior to changes in predation pressure in the absence of neutral genetic changes. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 6256–6260.

Decaestecker E., De Meester L. & Ebert D. (2002) In deep trouble: habitat selection constrained by multiple enemies in zooplankton. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 5481–5485.

Decaestecker E., Declerck S., De Meester L. & Ebert D. (2005) Ecological implications of parasites in natural *Daphnia* populations. *Oecologia*, **144**, 382–390.

Decaestecker E., Gaba S., Raeymaekers J.A.M., Stoks R., Van Kerckhoven L., Ebert D. *et al.* (2007) Host-parasite 'Red Queen' dynamics archived in pond sediment. *Nature*, **450**, 870–873.

Decaestecker E., Lefever C., De Meester L. & Ebert D. (2004) Haunted by the past: evidence for dormant stage banks of microparasites and epibionts of *Daphnia*. *Limnology and Oceanography*, **49**, 1355–1364.

Dieguez M.C. & Gilbert J.J. (2011) *Daphnia*-rotifer interactions in Patagonian communities. *Hydrobiologia*, **662**, 189–195.

Ebert D. (2005) *Ecology, Epidemiology, and Evolution of Parasitism in Daphnia [Internet]*. National Library of Medicine (US), National Center for Biotechnology, Bethesda, MD.

Ebert D. (2008) Host-parasite coevolution: insights from the *Daphnia*-parasite model system. *Current Opinion in Microbiology*, **11**, 290–301.

Frisch D., Morton P.K., Chowdhury R.P., Culver B.W., Colbourne J.K., Weider L.J. *et al.* (2014) A millennial-scale chronicle of evolutionary responses to cultural eutrophication in *Daphnia*. *Ecology Letters*, **17**, 360–368.

Gilbert J.J. (1988) Suppression of rotifer populations by *Daphnia* - a review of the evidence, the mechanisms, and the effects on zooplankton community structure. *Limnology and Oceanography*, **33**, 1286–1303.

Gilbert J.J. (2004) Females from resting eggs and parthenogenetic eggs in the rotifer *Brachionus calyciflorus*: lipid droplets, starvation resistance and reproduction. *Freshwater Biology*, **49**, 1505–1515.

Gilbert J.J. & Hampton S.E. (2001) Diel vertical migrations of zooplankton in a shallow, fishless pond: a possible avoidance-response cascade induced by notonectids. *Freshwater Biology*, **46**, 611–621.

Green J. (1974) Parasites and epibionts of Cladocera. *Transactions of the Zoological Society of London*, **32**, 417–515.

Hairston N.G., Lampert W., Caceres C.E., Holtmeier C.L., Weider L.J., Gaedke U. *et al.* (1999) Lake ecosystems - Rapid evolution revealed by dormant eggs. *Nature*, **401**, 446.

Iyer N. & Rao T.R. (1993) Effects of epizoic rotifer *Brachionus rubens* on the population growth of 3 Cladoceran species. *Hydrobiologia*, **255**, 325–332.

Jansen M., Stoks R., Decaestecker E., Coors A., Van Meutter De F. & De Meester L. (2010) Local exposure shapes spatial patterns in infectivity and community structure of Daphnia parasites. *Journal of Animal Ecology*, **79**, 1023–1033.

Jaramillo-Londono J.C. & Pinto-Coelho R.M. (2010) Interaction between *Hexarthra intermedia* (Rotifera) *Bosmina longirostris* (Cladocera): a case of opportunistic feeding or interference competition. *Journal of Plankton Research*, **32**, 961–966.

Jeppesen E., Jensen J.P., Søndergaard M., Lauridsen T. & Landkildehus F. (2000) Trophic structure, species richness and biodiversity in Danish lakes: changes along a phosphorus gradient. *Freshwater Biology*, **45**, 201–218.

Jeppesen E., Leavitt P., De Meester L. & Jensen J.P. (2001) Functional ecology and palaeolimnology: using cladoceran remains to reconstruct anthropogenic impact. *Trends in Ecology & Evolution*, **16**, 191–198.

Jeppesen E., Søndergaard M., Jensen J.P., Mortensen E., Hansen A.-M. & Jørgensen T. (1998) Cascading trophic interactions from fish to bacteria and nutrients after reduced sewage loading: an 18-year-study of a shallow hypertrophic lake. *Ecosystems* **1**, 250–267.

Kankaala P. & Eloranta P. (1987) Epizoic ciliates (*Vorticella sp*) complete for food with their host *Daphnia-longispina* in a small polyhumic lake. *Oecologia*, **73**, 203–206.

Kerfoot W.C. & Weider L.J. (2004) Experimental paleoecology (resurrection ecology): chasing Van Valen's Red Queen hypothesis. *Limnology and Oceanography*, **49**, 1300–1316.

Matveeva L.K. (1989) Interrelations of rotifers with predatory and herbivorous Cladocera - A review of Russian works. *Hydrobiologia*, **186**, 69–73.

May L. (1989) Epizoic and parasitic rotifers. *Hydrobiologia*, **186**, 59–67.

Michels H., Amsinck S.L., Jeppesen E. & De Meester L. (2007) Interclonal variation in diel horizontal migration behaviour of the water flea *Daphnia magna*-searching for a signature of adaptive evolution. *Hydrobiologia*, **594**, 117–129.

Pauwels K., De Meester L., Put S., Decaestecker E. & Stoks R. (2010) Rapid evolution of phenoloxidase expression, a component of innate immune function, in a natural population of *Daphnia magna*. *Limnology and Oceanography*, **55**, 1408–1413.

Penczykowski R.M., Forde S.E. & Duffy M.A. (2011) Rapid evolution as a possible constraint on emerging infectious diseases. *Freshwater Biology*, **56**, 689–704.

Preisser E.L., Bolnick D.I. & Benard M.F. (2005) Scared to death? The effects of intimidation and consumption in predator-prey interactions. *Ecology*, **86**, 501–509.

Pulkkinen K. & Ebert D. (2004) Host starvation decreases parasite load and mean host size in experimental populations. *Ecology*, **85**, 823–833.

Stearns S.C. (1992) *The Evolution of Life Histories*. Oxford University Press, New York.

Tessier A.J., Leibold M.A. & Tsao J. (2000) A fundamental trade-off in resource exploitation by *Daphnia* and consequences to plankton communities. *Ecology*, **81**, 826–841.

Threlkeld S.T., Chiavelli D.A. & Willey R.L. (1993) The organization of zooplankton epibiont communities. *Trends in Ecology & Evolution*, **8**, 317–321.

Verschuren D. & Marnell L.F. (1997) Fossil zooplankton and the historical status of westslope cutthroat trout in a headwater lake of Glacier National Park, Montana. *Transactions of the American Fisheries Society*, **126**, 21–34.

Willey R.L., Cantrell P.A. & Threlkeld S.T. (1990) Epibiotic euglenoid flagellates increase the susceptibility of some zooplankton to fish predation. *Limnology and Oceanography*, **35**, 952–959.

Wolinska J., Spaak P., Koerner H., Petrusek A., Seda J. & Giessler S. (2011) Transmission mode affects the population genetic structure of Daphnia parasites. *Journal of Evolutionary Biology*, **24**, 265–273.

Woolhouse M.E.J., Webster J.P., Domingo E., Charlesworth B. & Levin B.R. (2002) Biological and biomedical implications of the co-evolution of pathogens and their hosts. *Nature Genetics*, **32**, 569–577.

Yin M., Laforsch C., Lohr J.N. & Wolinska J. (2011) Predator-induced defense makes *Daphnia* more vulnerable to parasites. *Evolution*, **65**, 1482–1488.

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